U.S. Fish and Wildlife Service

# Evaluation of Larval Pacific Lamprey Occupancy in Portland Harbor Superfund Area Restoration Sites: Rinearson Natural Area

*2015 Annual Report*

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## Disclaimers

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*Abstract* –Within and around the Portland Harbor Superfund site on the Willamette River, habitat restoration actions focused on juvenile salmonids including Chinook salmon *Oncorhynchus tshawytscha* are being implemented which may also have effects on co-occurring Pacific lamprey *Entosphenus tridentatus*. Use of restored habitats by lampreys, particularly the larval life stage has not been extensively studied. As such, there is interest in monitoring the effectiveness of the restoration, in part, relative to larval Pacific lamprey as well as learning more about larval lamprey habitat preferences and use of different habitats. Determining the effects of restoration actions on Pacific lamprey requires evaluation of lamprey occurrence before and after project implementations. We evaluated occupancy, detection, and habitat use of larval Pacific lamprey and *Lampetra* spp. at confluence habitats (within the Willamette River mainstem) as well as within tributary habitats at the Rinearson Natural Area restoration site and a reference site, Cemetery Creek. A generalized random-tessellation stratified (GRTS) approach was used to delineate sample units, quadrats (30 m x 30 m square) within mainstem confluence areas and sample reaches (50 m long) within tributary habitats, in a random, spatially balanced order. Mainstem quadrats and tributary reaches were sampled for larvae by electrofishing. Both the Rinearson Natural Area restoration site and the Cemetery Creek reference site were occupied by larval Pacific lamprey. At the Rinearson Restoration site, larval lampreys were detected in 3 of 10 confluence quadrats sampled in the Willamette River, and one of seven tributary reaches sampled in Rinearson Creek. Detection probabilities at the Rinearson Natural Area were $d = 0.3$ in confluence quadrats and $d = 0.14$ in tributary reaches. At the Cemetery Creek reference site larval lampreys were detected in 5 of 10 confluence quadrats sampled, and zero of two tributary reaches sampled. Detection probabilities at the reference site were $d = 0.5$ in confluence quadrats and $d = 0$ in tributary reaches. Although larval Pacific lampreys were detected within a tributary reach in Rinearson Creek, the detection occurred approximately 30 m from the confluence with the Willamette River in habitat that appeared to be influenced by backwater from the Willamette River. Thus, it is likely the larvae collected in Rinearson Creek had washed in from the Willamette River and were not produced within Rinearson Creek. This information will serve as a baseline for monitoring and evaluation of larval lamprey occupancy in the Rinearson Natural Area pre- and post- habitat restoration actions at the site.

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# Introduction

Pacific lamprey *Entosphenus tridentatus* in the Columbia River Basin (CRB) and other areas have experienced a great decline in abundance (Close et al. 2002) and have been given protected status within Oregon (Kostow 2002). Lamprey are culturally important to Native American tribes, are ecologically important within the food web, and are an indicator species whose decline provides further insight into the impact of human actions on ecological function (Close et al. 2002). Much information is lacking on the basic biology, ecology, and population dynamics that is required for effective conservation and management.

Pacific lampreys have a complex life history that includes a multiple year larval (ammocoete), migratory juvenile (macrophthalmia), and adult marine phase (Scott and Crossman 1973). Larvae and juveniles are strongly associated with stream and river sediments. Larvae live burrowed in stream and river sediments for multiple years after hatching, where they filter feed detritus and organic material (Sutton and Bowen 1994). Larvae metamorphose into juveniles from July to December (McGree et al. 2008) and major migrations are made downstream to the Pacific Ocean in the spring and fall (Beamish and Levings 1991). The sympatric western brook lamprey *Lampetra richardsoni* does not have a major migratory or marine life stage although adults may locally migrate upstream before spawning (Renaud 1997). For both species, the majority of the information on distribution and habitat preference of larvae comes from CRB tributary systems (Moser and Close 2003; Torgersen and Close 2004; Stone and Barndt 2005; Stone 2006) and coastal basins (Farlinger and Beamish 1984; Russell et al. 1987; Gunckel et al. 2009).

Larval lamprey are known to occur in sediments of low-gradient streams (<5$^{th}$ order [1:100,000 scale]; Torgersen and Close 2004) but their use of larger river habitats in relatively deeper areas is less known. Downstream movement of larvae, whether passive or active, occurs year-round (Nursall and Buchwald 1972; Gadomski and Barfoot 1998; White and Harvey 2003). Sea lamprey *Petromyzon marinus* ammocoetes have been documented in deepwater habitats in tributaries of the Great Lakes, within the lakes in proximity to river mouths (Hansen and Hayne 1962; Wagner and Stauffer 1962; Lee and Weise 1989; Bergstedt and Genovese 1994; Fodale et al. 2003), and in the large, connecting St. Marys River (Young et al. 1996). However, references to other species occurring in deepwater or lacustrine habitats are scarce (American brook lamprey *L. appendix*; Hansen and Hayne 1962). In the Pacific Northwest, observations of larval lamprey occurrence in large rivers have been made, for example during smolt monitoring operations at Columbia River hydropower facilities, impinged on screens associated with juvenile bypass systems (Moursund et al. 2003; CRITFC 2008), or through observation during dewatering events. Specific collections of ammocoetes have been made in large river habitats in British Columbia which are thought to be representative of downstream migrating ammocoetes (Beamish and Youson 1987; Beamish and Levings 1991). More recently, evaluations of larval Pacific lamprey occupancy and distribution in mainstem river habitats have suggested widespread occurrence in certain areas of the Columbia River and Willamette River mainstem (Jolley et al. 2012; Jolley et al. 2013, Jolley et al. 2014)

A portion of the mainstem of the lower Willamette River that is known to be occupied by larval Pacific and western brook lamprey (Jolley et al. 2012) was declared a Superfund Site in 2000 by the U.S. Environmental Protection Agency. The Superfund study area extends from river kilometer 3.2 to river kilometer 18.9 and has a broader focus area extending from the

Columbia River to Willamette Falls (Figure 1). To mitigate for past environmental damage being identified through the Natural Resource Damage Assessment (NRDA) process, this area is subject to various restoration activities as well as assessments of the effectiveness of any restoration. Presently, aquatic restoration projects are focused on restoring juvenile Chinook salmon *Oncorhynchus tshawytscha* habitat. It is unclear whether any of the restoration activities will provide additional benefits to other co-occurring species including larval and juvenile Pacific lamprey that may likewise occur in these areas. However, these activities provide an opportunity to understand the potential effects of habitat restoration on larval and juvenile lampreys. As such, there is interest in monitoring the effectiveness of the restoration, in part, relative to larval Pacific lamprey.

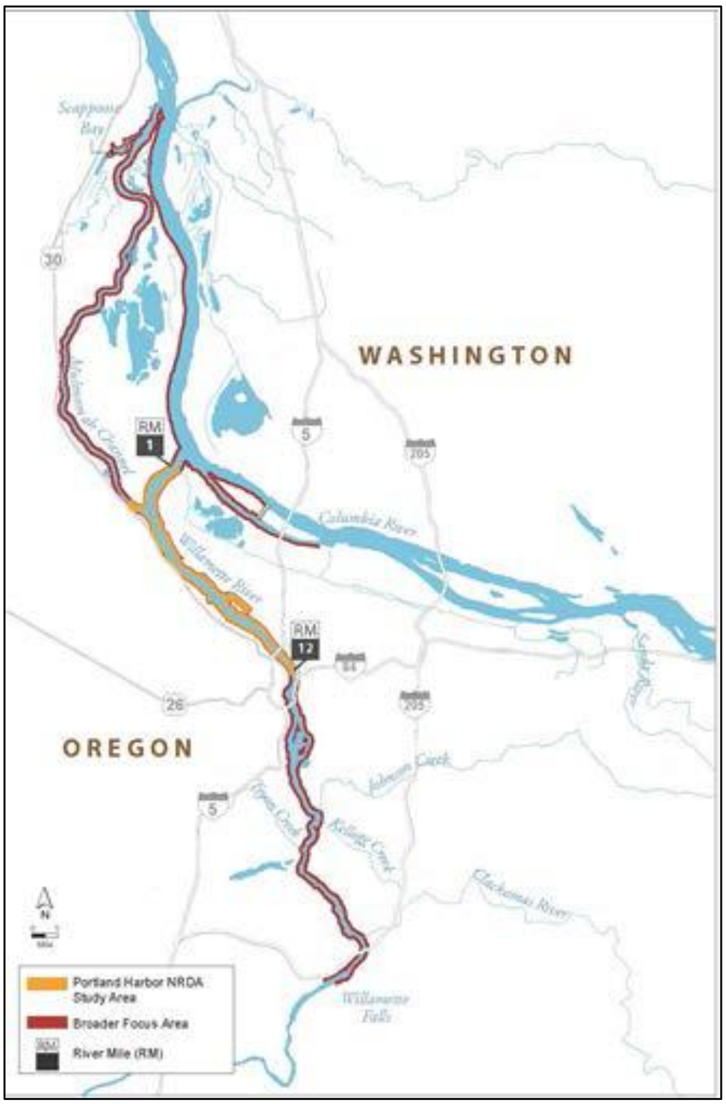


**Figure 1. Portland Harbor Superfund study area (orange outline) and the broader focus area (red outline) on the lower Willamette River.**

A lamprey monitoring plan (LMP) for restoration projects in the Portland Harbor Superfund area was developed based on a set of monitoring goals and objectives that were identified by the Trustee Council and lamprey biologists over two workshops held in the fall of 2011. The LMP priorities included (i.) monitoring the impact of restoration actions on larval and juvenile lamprey populations and health in Portland Harbor, and (ii.) gathering information about larval and juvenile lamprey life history, biology, and habitat requirements that could be used by the Trustee Council to inform future design and evaluation of lamprey restoration projects. Since lamprey biology and life history are different from other aquatic biota, the overlap between the LMP and the general restoration monitoring and stewardship plan is not extensive. The LMP differs from the general restoration monitoring and stewardship plan, in part, because the lamprey monitoring is proposed to continue for a period of 20 years. In most cases, the metrics proposed for collection as part of the lamprey monitoring effort need to be co-located with lamprey sampling. To maximize efficiencies, the Trustee Council will, to the extent possible, use data collected as part of the LMP for general restoration monitoring and stewardship. Biologists recommended monitoring lamprey for 20 years, with the goal of capturing data for 1 to 2 complete generations. Pre-implementation monitoring will be conducted to the extent practical at each restoration site. Lampreys are expected to colonize habitats rapidly. Therefore, monitoring will be conducted on a yearly basis for the first five years, and every five years thereafter. In general, the proposed work is guided by the LMP. However, due to site specific conditions and constraints, the specific metrics and timing of monitoring proposed for any given site may differ slightly from those outlined in the LMP.

In 2015, we began to investigate and document patterns of larval lamprey occupancy, distribution, and habitat use in or near the Rinearson Natural Area restoration site on the lower Willamette River. Understanding larval lamprey usage of habitats in and adjacent to restoration sites is critical to gauging the effectiveness of restoration activities. At present, little specific information is available on whether lampreys colonize restored habitats, which life stages may use these habitats, or how quickly and for how long they use these habitats. A before-after control-impact (BACI) approach will be used to evaluate the effectiveness of restoration activities, as that allows us to make inferences about whether changes in lamprey occupancy observed at the restoration site are the result of the restoration actions. Thus, we propose to determine whether larval Pacific lamprey occupy restoration sites and reference sites both prior to and after restoration actions. Our specific objectives for this phase of NRDA restoration monitoring are as follows:

1. Determine whether lampreys occupy the Rinearson Natural Area restoration site and the Cemetery Creek reference site.
2. Determine the types of habitat available at each site and in which habitat types lamprey are detected.
3. Characterize lamprey species and life history stage that occupy each site.
4. Evaluate the health of lamprey detected at each site.

## Study Sites

*Restoration Site*

Rinearson Creek flows through the Rinearson Natural Area restoration site (Clackamas County, OR) and enters the Willamette River from the east, just downstream of the mouth of the Clackamas River (river km 39; Figure 2). Currently the site has tributary or slough habitat that drains into the Willamette River, as well as associated confluence habitat in the mainstem Willamette River. Larval lamprey are known to occur in the mainstem of the Willamette River in this region (Jolley et al. 2012), and have access to and the potential to occur in proposed restoration areas in Rinearson Creek and confluence habitats in the mainstem Willamette River. Pre-restoration monitoring consisted of sampling for larval lamprey in tributary or slough reaches in Rinearson Creek as well as confluence habitats in the mainstem Willamette River.

*Reference Site*

Cemetery Creek (Multnomah County, OR) was selected as a reference site because it is similar in size and located in proximity to the Rinearson Natural Area restoration site. Cemetery Creek enters the Willamette River from the west, upstream of Ross Island (river km 27; Figure

2). The Cemetery Creek reference site has tributary or slough habitat that drains into the Willamette River, as well as associated confluence habitat in the mainstem Willamette River. Larval lamprey are known to occur in the mainstem of the Willamette River in this region (Jolley et al. 2012), and have access to and the potential to occur in Cemetery Creek and confluence habitats in the mainstem Willamette River. Pre-restoration monitoring at the Cemetery Creek reference site consisted of sampling for larval lamprey in tributary or slough reaches in Cemetery Creek as well as confluence habitats in the mainstem Willamette River.

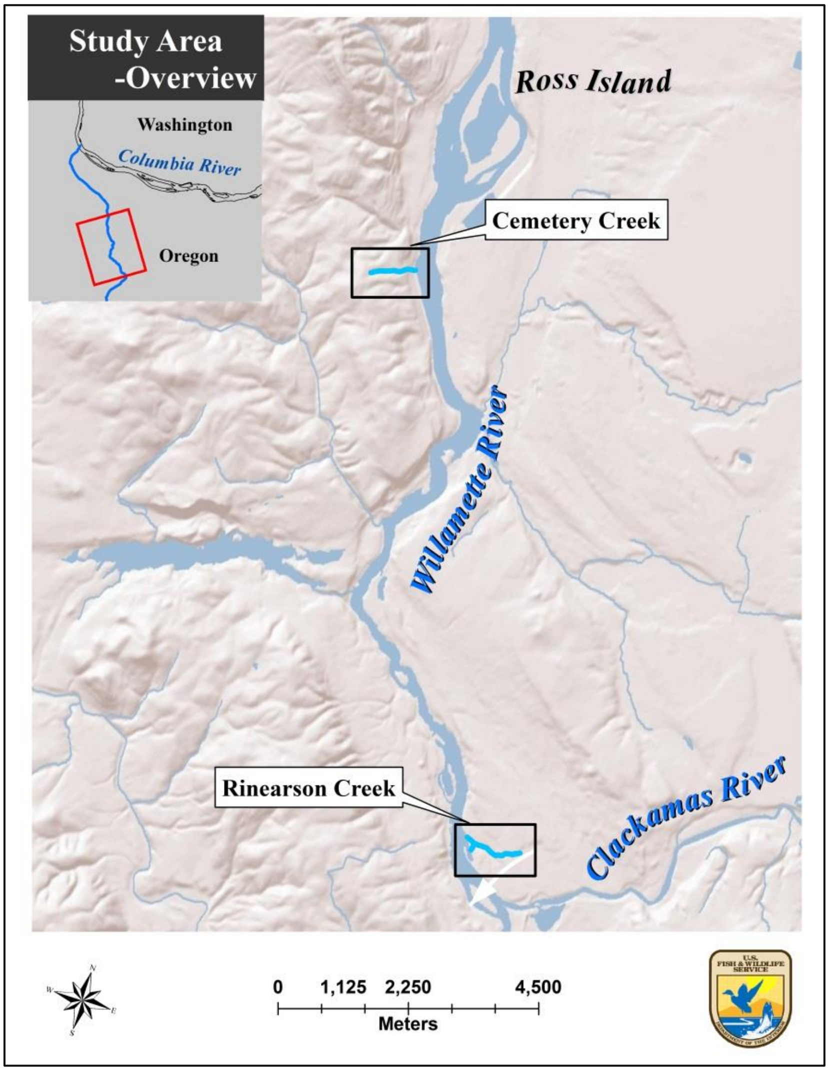


**Figure 2. Locations of the Rinearson Natural Area restoration site and Cemetery Creek reference site along the lower Willamette River. Rinearson Creek (river km 39) enters the Willamette River just downstream of the Clackamas River confluence. Cemetery Creek (river km 27) enters the Willamette River just upstream of Ross Island near downtown Portland.**

# Methods

**Sample Framework**

We evaluated occupancy of larval lamprey in the restoration and reference sites by adapting an approach that has been applied previously to studies of larval lamprey occupancy in the Columbia River basin in both mainstem and tributary habitats (Silver et al. 2010; Jolley et al. 2012; Jolley et al. 2013; Jolley et al. 2014; USFWS unpublished data). The approach has several requirements: 1) a unit- and gear-specific detection probability (assumed or estimated); 2) the probability of presence (given no detection) at a predetermined acceptably low level; and 3) random identification of spatially balanced sample units that allow estimation of presence and refinement of detection probabilities. A unit-specific probability of detection, $d_{unit}$, was calculated as the proportion of sample quadrats or reaches in which larvae were captured. The posterior probability of area occupancy, given a larval lamprey was not detected, was estimated as:

$$(1)\ \mathrm{P}(F|C_o) = \frac{\mathrm{P}(C_o|F)\cdot\mathrm{P}(F)}{\mathrm{P}(C_o|F)\cdot\mathrm{P}(F) + \mathrm{P}(C_o|{\sim}F)\cdot\mathrm{P}({\sim}F)},$$

where P($F$) is the prior probability of larval lamprey presence. Although in this case we knew the lower Willamette River was occupied with larval lamprey, a P($F$) of 0.5 (uninformed) was used for future study design (i.e., P[$F|C_o$] ) in areas where larval lamprey presence is unknown. P($\sim F$), or 1 – P($F$), is the prior probability of species absence, and P($C_o|F$), or 1 – $d$, is the probability of not detecting a species when it occurs ($C_0$ = no detection; Peterson and Dunham 2003). Random identification of spatially balanced sample units was achieved by using a generalized random-tessellation stratified (GRTS) approach to delineate sample units in an ordered, unbiased manner (Stevens and Olsen 2004). Patterns of occupancy by area were compared using the Fisher's Exact test for differences in detection probabilities. Significance levels were set at $\alpha = 0.05$

*Confluence Area Methods*

Confluence area quadrats at both the restoration and reference sites were delineated using the generalized random-tessellation stratified (GRTS) approach scripted in Program R (Stevens and Olsen 2004; Jolley et al. 2012; R Core Team, 2013). The GRTS method assigns a hierarchical order to quadrats which can be used as an unbiased method of ranking the priority of quadrats for sampling. Delineation of quadrats that are unbiased, randomly selected, and spatially balanced within a sample universe allows for calculation of unit-specific detection probabilities. In turn, unit-specific estimates of detection probability can be applied to determine sample effort necessary for achieving a desired level of certainty that an area is not occupied by lamprey when they are not detected. Here we proposed to use a sampling effort (number of sample quadrats) that we estimate would allow for at least 80% certainty that larval lampreys do not occupy at least 20% of a confluence area when they are not detected (see Bayley and Peterson 2001; Peterson and Dunham 2003). The amount of effort was based, in part, on estimates of quadrat-specific detection probabilities generated from previous work (Jolley et al. 2012). Sample effort was also dependent, in part, on total area. In the case of both the Rinearson Natural area restoration site and the Cemetery Creek reference site, this sample effort corresponded to sampling of 10 confluence quadrats at each location.

Confluence quadrats at the restoration and reference sites were selected from a layer of quadrats delineated and overlaid on the lower Willamette River from Willamette Falls to the Columbia River in association with previous lamprey research in this region (Jolley et al. 2012). At each creek confluence area, a subset of quadrats from the lower Willamette River layer was filtered according to a 100 m semicircular buffer centered on the confluence of each creek and the Willamette River (Figure 3). Because Rinearson Creek forks into two distributary channels near its confluence with the Willamette River, the confluence quadrat selection process was duplicated at each of the two distinct confluence areas (Figure 3). The selection process resulted in a total of 34 quadrats at the Rinearson Creek confluence areas, of which the 10 lowest numbered quadrats as ordered by the GRTS method were assigned the highest priority for sampling. Given the two distinct confluence areas of Rinearson Creek, the sample effort of 10 quadrats was divided among the two locations, with five quadrats being sampled at each confluence area. At the Cemetery Creek confluence area, the selection process produced a total of 17 quadrats (Figure 3), of which the 10 lowest numbered quadrats as ordered by the GRTS method were assigned the highest priority for sampling.

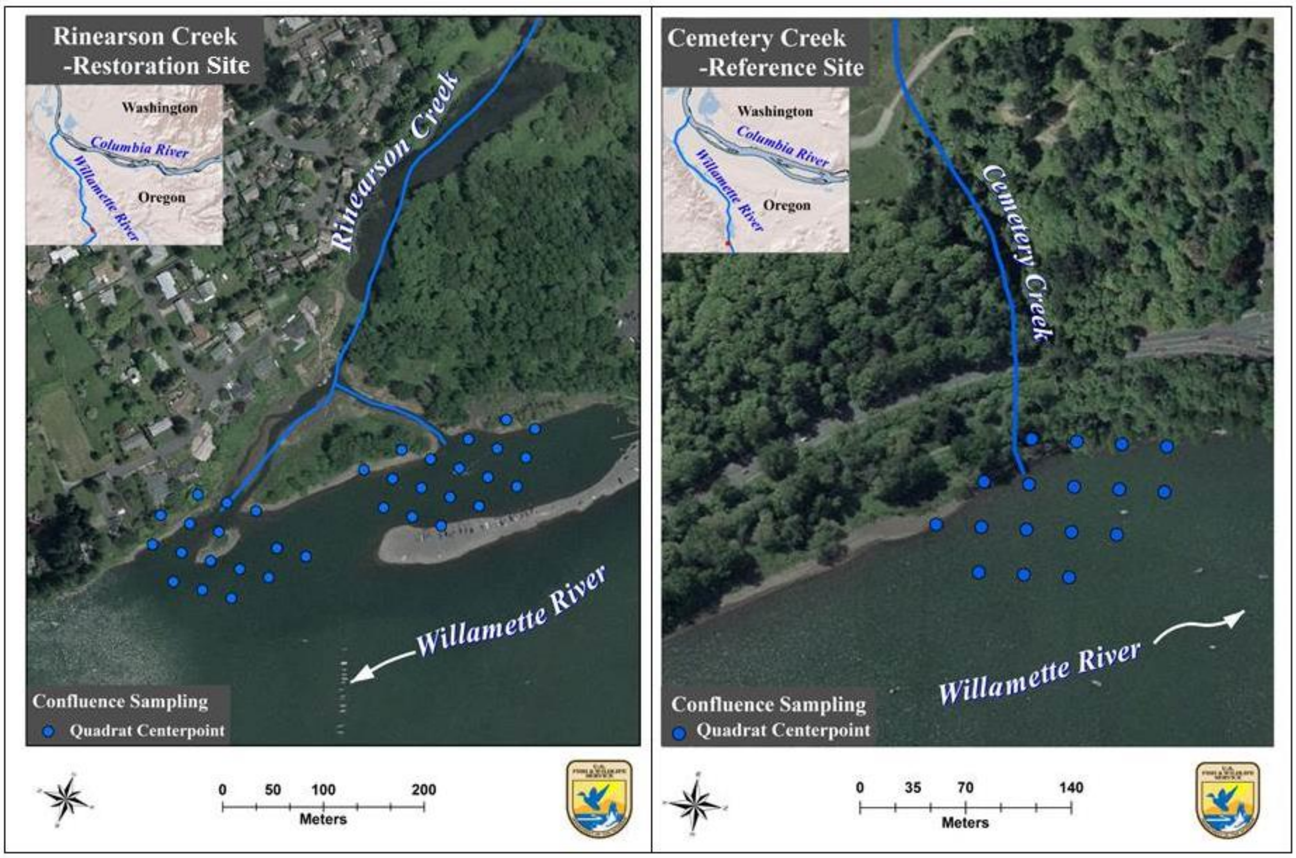


**Figure 3. Sample quadrats (blue points represent quadrat center points) in confluence areas at the restoration and reference sites were selected within a 100 m semicircular radius centered on the intersection of Rinearson Creek (above left; river km 39) and Cemetery Creek (above right; river km 26) and the Willamette River. From the available quadrats, the 10 lowest numbered quadrats as ordered by the GRTS method at each tributary location were assigned the highest priority for sampling.**

Each sampling event consisted of a single drop with deepwater electrofishing equipment within the 30 m by 30 m quadrat (Bergstedt and Genovese 1994; Jolley et al. 2012). Quadrats were accessed and sampled by boat, using quadrat center point Universal Transverse Mercator (UTM) coordinates for navigation. When quadrats could not be sampled due, for example, to dewatered conditions, depth less than 0.3 m, excessive velocity, or excessive depth (>21 m) they were eliminated and subsequent quadrats were increased in priority (Table 1). The deepwater electrofisher was comprised of a modified AbP-2 electrofisher (ETS Engineering, Verona, WI) which delivered electrical stimulus to river bottom substrates at electrodes mounted to a fiberglass bell (or hood; 0.61 $m^2$ in area). The electrofisher delivered three pulses DC per second at 10% duty cycle, with a 2:2 pulse train (i.e., two pulses on, two pulses off). Output voltage was adjusted at each quadrat to maintain a peak voltage gradient between 0.6 and 0.8 V/cm across the electrodes. The electrofisher bell was coupled by a 3" vinyl suction hose to a gasoline-fueled hydraulic pump. The hydraulic pump was started approximately 5 seconds prior to shocking to purge air from the suction hose. Suction was produced by directing flow from the pump through a hydraulic eductor, which allows larvae to be collected in a mesh basket (27 x 62 x 25 cm; 2 mm wire mesh) while preventing them from passing through the pump. A 60 second pulse delivery was followed by an additional 60 seconds of pumping to further allow displaced larvae to cycle through the hose and into the collection basket. The sampling techniques are described in detail by Bergstedt and Genovese (1994) and were similar to those used in the Great Lakes region (Fodale et al. 2003) and the Willamette River (Jolley et al. 2012).

### *Tributary/Slough Area Methods*

Evaluation of larval lamprey occupancy of tributary habitats was conducted in Rinearson Creek at the restoration site and Cemetery Creek at the reference site. In Rinearson Creek, sampling occurred over an approximately 1200 m long segment of creek, spanning from the confluence with the Willamette River upstream to the crossing of River Road (Milwaukie, OR). In Cemetery Creek, the tributary area of interest was less than 400 m in length, spanning from the confluence with the Willamette River upstream approximately 300 m to a reach of very high gradient. Here we proposed to use a sampling effort (number of sample reaches) that would allow for at least 80% certainty that larval lampreys do not occupy at least 20% of a tributary area when they are not detected (see Bayley and Peterson 2001; Peterson and Dunham 2003). The amount of effort was based, in part, on estimates of reach-specific detection probabilities generated from previous work (Silver et al. 2010; USFWS unpublished data). Sample effort was also dependent, in part, on total area. At the restoration site, the area of interest in Rinearson Creek was longer than 400 m, thus we proposed to sample seven 50 m GRTS reaches in Rinearson Creek. At the reference site, the area of interest in Cemetery Creek was less than 400 m in length, thus we proposed to sample all viable reaches (contiguous 50 m reaches) in Cemetery Creek up to a total of 350 m (Figure 4).

Delineation of random spatially balanced 50 m sample reaches in Rinearson Creek was again accomplished using a generalized random-tessellation stratified (GRTS) approach scripted in Program R (Stevens and Olsen 2004; R Core Team 2013). The GRTS method assigns a hierarchical order to the reaches within the creek which is used as an unbiased method of ranking the priority of reaches for sampling. Delineation of sample reaches that are unbiased, randomly selected, and spatially balanced within a sample universe allows for calculation of unit-specific detection probabilities. In turn, unit-specific estimates of detection probability can be applied to determine sample effort necessary for achieving a desired level of certainty that a tributary is not occupied by lamprey when they are not detected. In Rinearson Creek, sample reaches were delineated at a rate of one 50 m reach for every 50 m of stream. Thus, within the approximately 1200 m long study area in Rinearson Creek, 24 sample reaches were delineated, of which the lowest numbered seven reaches as ordered by the GRTS method were assigned the highest priority for sampling (Figure 4).

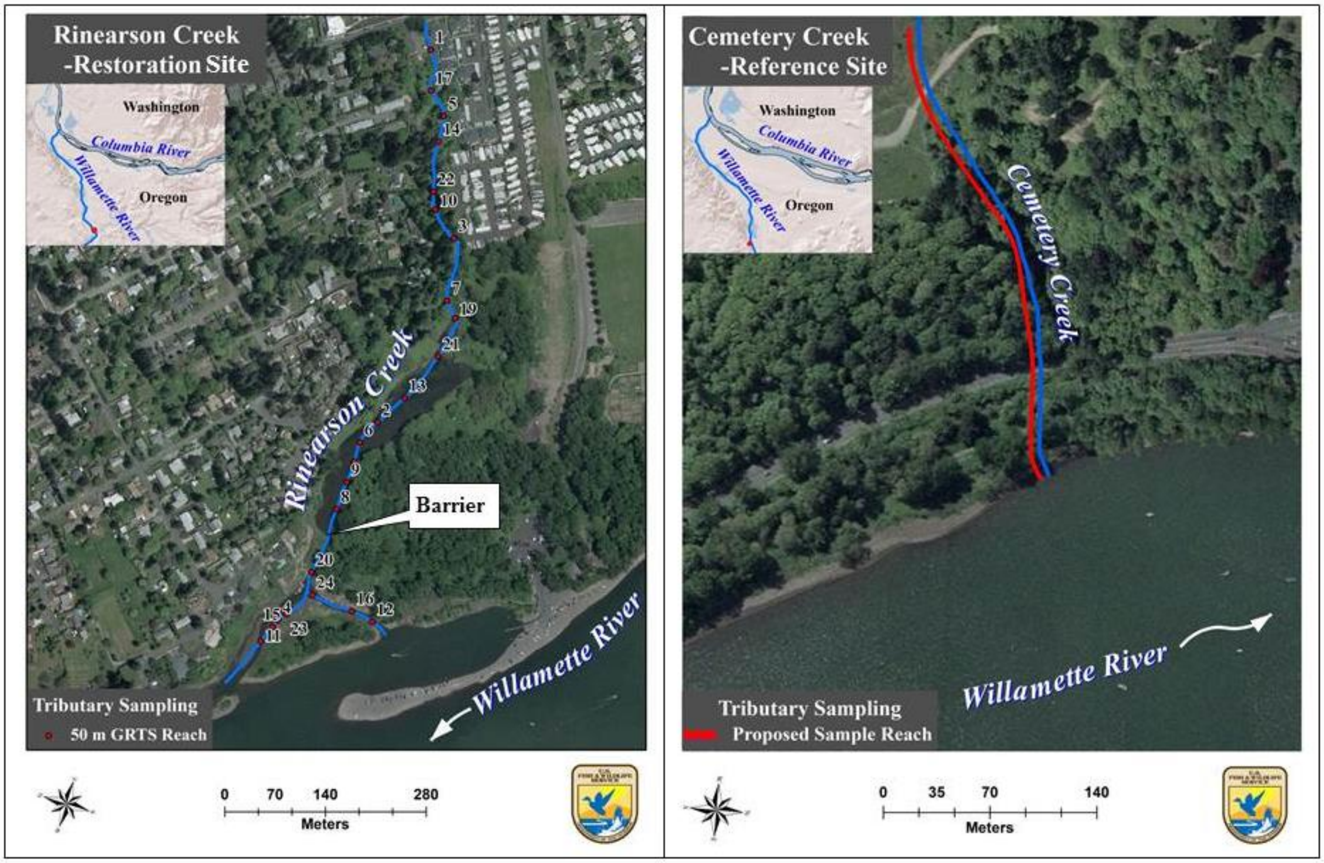


**Figure 4. Tributary sample reaches (red points represent downstream reach boundary) in Rinearson Creek (above left) as delineated by the GRTS method. The lowest numbered seven reaches were assigned the highest priority for sampling. The tributary sample reach in Cemetery Creek (above right; red line) was less than 350 m and so the entire reach was proposed for sampling.**

For tributary or slough (wadeable) areas, each sampling event consisted of electrofishing reaches for larval lamprey (Silver et al. 2010). Sample reaches were accessed on foot using GPS units loaded with sample reach UTMs for navigation. When a reach could not be sampled due, for example, to dewatered conditions, excessive depth (> 2 m), or lack of access due to private property, they were eliminated and subsequent reaches were increased in priority. Once a sample reach was accessed, a 50 m segment was measured and flagged. Water temperature and conductivity were recorded in each reach. The reach was electrofished using an AbP-2 backpack electrofisher. Power output settings for the AbP-2 were adapted from Weisser and Klar (1990). Initially, the electrofisher delivered three DC pulses per second at 25% duty cycle, 125 V, with a 3:1 burst pulse train (i.e., three pulses on, one pulse off). This current is designed to stimulate burrowed ammocoetes to enter the water column. Once a larva was observed in the water column, 30 pulses/second were applied to temporarily immobilize the larva for capture in a net.

We spent relatively more time within each reach electrofishing areas of preferred larval lamprey rearing habitat where depositional silt and sand substrates were dominant (henceforth Type I habitat, Slade et al. 2003). Relatively less time was spent electrofishing areas with hard bedrock and boulder substrates. All larval lamprey observed were captured and placed in buckets containing stream water.

### Biological Data Collection

Collected lamprey were anesthetized in a solution of buffered tricaine methanesulfonate (MS-222), measured for total length (TL in mm; total weight was not measured), classified according to developmental stage (i.e., ammocoete, macrophthalmia, or adult), and when possible (i.e., larvae > 60 mm TL; Goodman et al. 2009) identified to genus (i.e., *Entosphenus* [Pacific lamprey] or *Lampetra* [western brook or river lamprey]) according to visual evaluations of caudal fin pigmentation patterns. Caudal fin tissue samples were also collected for potential future assignment of genus genetically (Spice et al. 2011; Docker et al. *in review*). Tissue samples are archived at the Columbia River Fisheries Program Office (CRFPO) pending funding availability for genetic identification. Upon resuming active swimming behavior, larvae were released near the area of capture. Physical anomalies (lesions, suspected bird strikes, tumors, etc.) were recorded for all larvae. If abnormalities were observed on a larva, the individual would be euthanized and preserved for potential evaluation at a later date. In addition, observations of juveniles, adults, or suspected Pacific lamprey nests were also recorded.

### Habitat Data Collection

*Confluence Areas*

Concurrent to each sampling event a sediment sample was taken (when possible) from each quadrat with a Ponar bottom sampler (16.5 cm x 16.5 cm). Each sample was mixed thoroughly and approximately two, 250-500 ml subsamples were transferred to containers provided by a contracted laboratory. Samples were labeled with the site number, replicate number and date, placed on ice, returned to the USFWS office, and subsequently handled per the instructions provided from the contracted laboratory. Water temperature (°C), conductivity ($\mu$S/cm) and water depth were also measured at each quadrat. All confluence habitat variables are presented as mean (± s.e.) unless otherwise noted.

*Tributary/Slough Areas*

Sediment samples were collected from each 50 m sample reach. Samples were mixed thoroughly and approximately two, 250-500 ml subsamples were transferred to containers provided by a contracted laboratory. Each sample was labeled with the reach number, replicate number and date, placed on ice, returned to the USFWS office, and subsequently handled per the instructions provided from the contracted laboratory.

Within each sample reach, water temperature (°C) and conductivity ($\mu$S/cm) were measured, and visibility was qualitatively ranked as good, fair, or poor. The proportion (%) of Type 1 burrowing substrate within each reach was estimated. In general, larval lamprey habitats are classified as Type I, II, or III, and it is widely accepted that larvae appear to most prefer Type

I and least prefer Type III (see Slade et al. 2003). All tributary habitat variables are presented as mean (± s.e.) unless otherwise noted.

## Results

*Confluence Areas*

We sampled 10 of 13 confluence quadrats visited at the Rinearson Natural Area restoration site and 10 of 10 confluence quadrats visited at the Cemetery Creek reference site (Table 1). The feasibility of being able to sample a quadrat in each location was 77% and 100%, respectively. Quadrats that were not sampled were omitted because they were not feasible (dewatered conditions). At Rinearson Natural Area, larval lampreys (n = 6) were detected in 3 of 10 confluence quadrats ($d = 0.3$; Figure 5). At Cemetery Creek, larval lampreys (n = 8) were detected in 5 of 10 confluence quadrats ($d = 0.5$; Figure 5). The total number of larvae occupying any individual quadrat ranged from 0 to 2; no other life stages were detected at either location. Detection probabilities ($d$) did not differ between Rinearson Natural Area and Cemetery Creek sample sites (Fisher's Exact Test; $P = 0.65$).

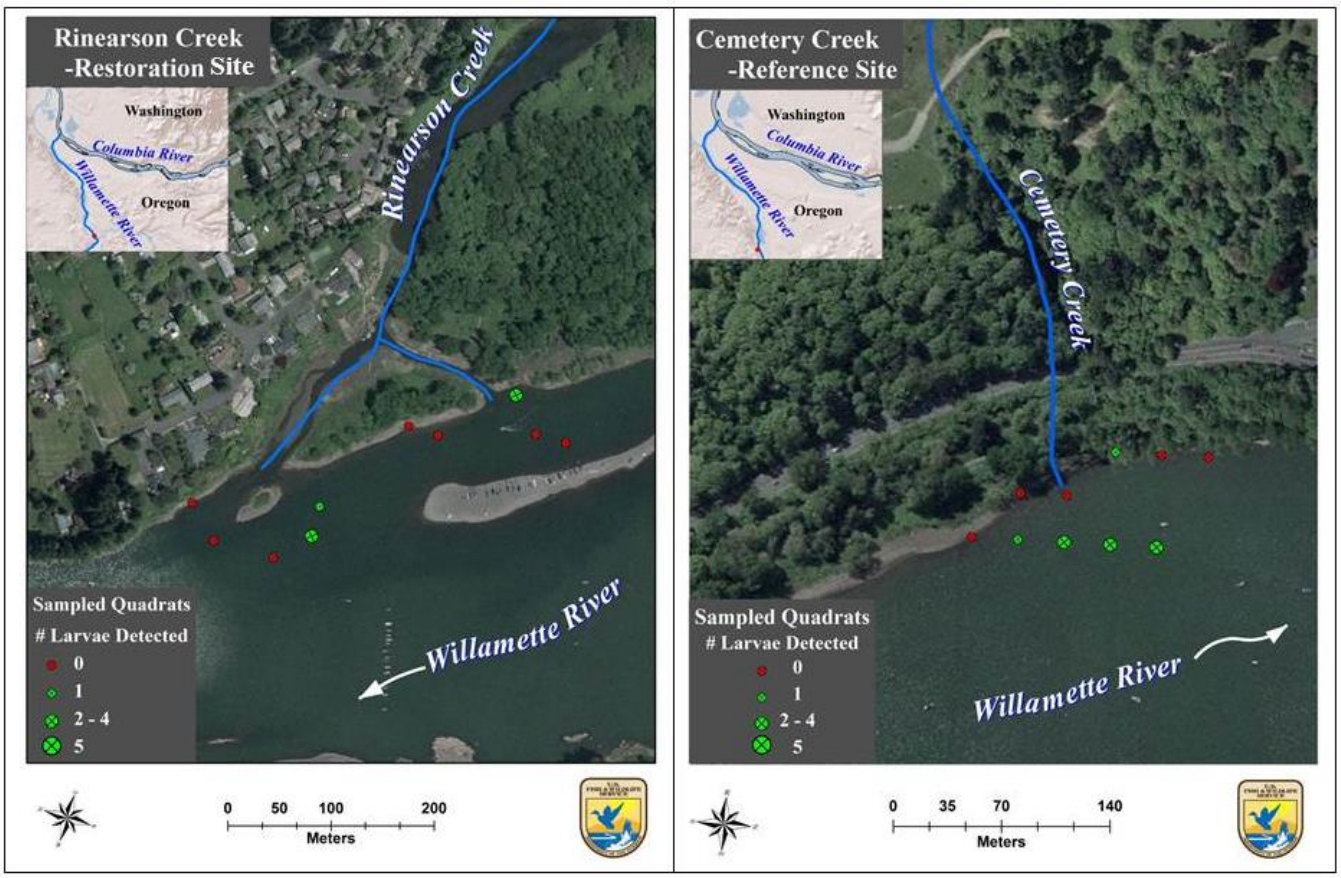


**Figure 5. Larval lampreys were detected in 3 of 10 confluence quadrats sampled in the mainstem Willamette River at the Rinearson Natural Area restoration site (above left), and 5 of 10 confluence quadrats sampled at the Cemetery Creek reference site (above right). Green points represent quadrats where larvae were detected, while red points represent quadrats where larvae were not detected.**

Of the six larvae collected at Rinearson Natural Area confluence quadrats, three were identified morphologically as Pacific lamprey, while three were too small to accurately identify visually (TL range 43 mm to 101 mm; Table 1; Figure 6). Of the eight larvae collected at Cemetery Creek confluence quadrats, two were identified morphologically as Pacific lamprey, while six larvae were too small to accurately identify visually (TL range 28 mm to 79 mm; Table 1; Figure 6). Larvae less than 40 mm TL are likely age-0 or age 1 while larger larvae are likely older, although definitive estimates of age based on size are difficult (Meeuwig and Bayer 2005). All collected larvae were in good condition and no visible external abnormalities were observed.

**Table 1. Total number of quadrats delineated, visited, sampled, and occupied and larval species present in 2015. Unidentified lamprey are noted as "UNID".**

| | | Quadrats | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Site | Date | Total | Visited | Sampled | Occupied | *d* | Pacific lamprey | *Lampetra* spp. | UNID | Total |
| Rinearson Confluence | 14-May | 34 | 13 | 10 | 3 | 0.3 | 3 | 0 | 3 | 6 |
| Cemetery Confluence | 27-May | 17 | 10 | 10 | 5 | 0.5 | 2 | 0 | 6 | 8 |

At Rinearson Natural Area confluence quadrats, sample depths ranged from 0.6 m to 7.3 m, and larvae were detected in depths from 1.7 m to 7.3 m. At Cemetery Creek confluence quadrats, sample depths ranged from 0.2 m to 6.0 m, and larvae were detected in depths from 0.7 m to 6.0 m. At Rinearson Natural Area, water temperature was 16.6°C (± 0.7) and conductivity was 90.7 $\mu$S/cm (± 1.4). At Cemetery Creek, water temperature was 17.3°C (± 0.2) and conductivity was 95.7 $\mu$S /cm (± 0.1). Sediment samples collected at each confluence quadrat were transferred to ALS Environmental Laboratory (Kelso, WA) in May 2015 for quantification of parameters including grain size, grain type, and organic content. Results of sediment analyses are in Appendix 1 below.

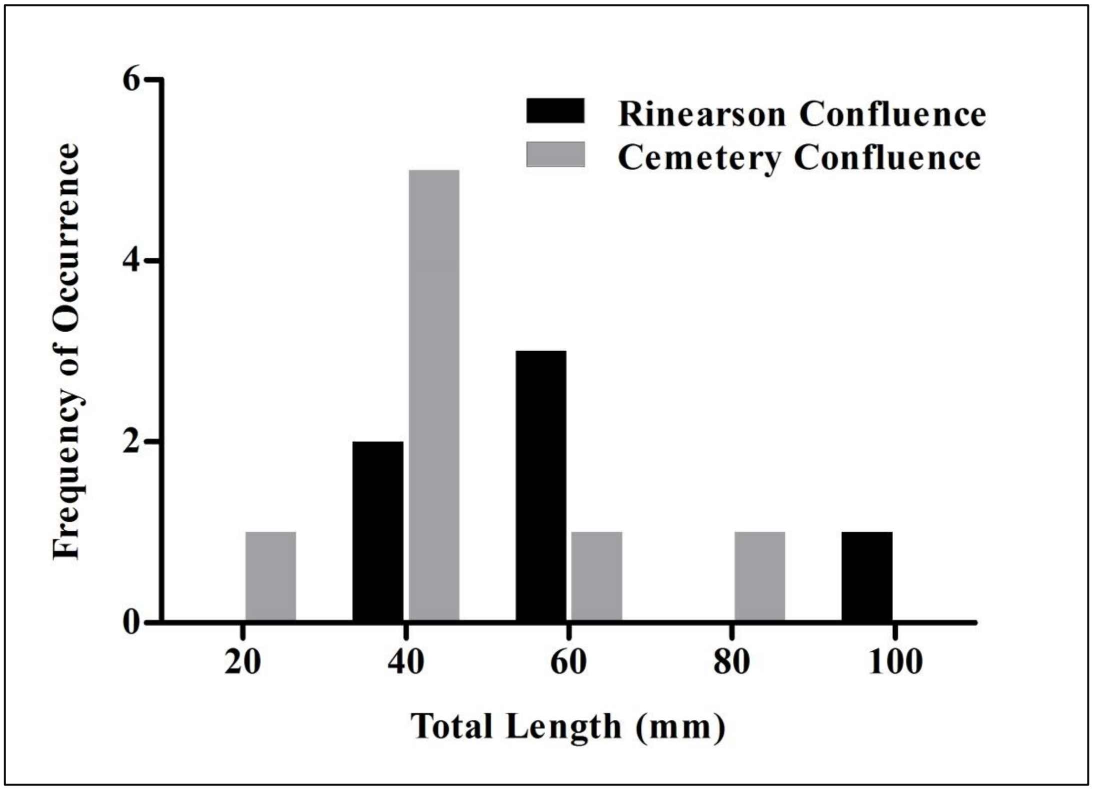


**Figure 6. Length-frequency (total length in 20 mm bins centered on values shown on x-axis) of larval lamprey collected at Rinearson Natural Area and Cemetery Creek confluence quadrats. Lamprey smaller than 60 mm were unidentified species and lamprey 60 mm or larger were morphologically identified as Pacific lamprey.**

*Tributary/Slough Areas*

In tributary habitats within Rinearson Creek, seven 50 m GRTS reaches were sampled from the confluence with the Willamette River upstream to the River Road crossing. We detected larval Pacific lampreys (n = 3; 106 mm, 117 mm, and 123 mm TL) in one of seven reaches sampled ($d$ = 0.14; Figure 7). Water temperature was 14.6°C (± 0.4), conductivity was 177.4 $\mu$S/cm (± 5.8), and % type 1 substrate was 77% (± 13) in sampled reaches. In the one reach occupied by larvae, 100% of the substrate in the reach was classified as type 1. In the 6 reaches not occupied by larvae 73% (± 15) of the substrate on average was classified as type 1. Larvae detected in the Rinearson Creek tributary reach were in the lowermost reach sampled in the creek, and occurred approximately 30 m from the confluence with the Willamette River (Figure 7) in an area that lacked flowing water and appeared to be influenced by backwater from the Willamette River (Figure 8). Five of the seven reaches were located above a water control structure (Figure 7; Figure 9) that is likely a barrier to all upstream (and downstream) fish migration in the creek. Resident (i.e., non-migratory) western brook lampreys could potentially have occurred in the creek prior to the construction of the barrier and persisted upstream of the impounded area, however no western brook lamprey were detected in the five reaches sampled above the barrier.

In Cemetery Creek, contiguous 50 m tributary reaches beginning at the Willamette River and continuing upstream approximately 300 m were proposed for sampling. We sampled approximately two contiguous 50 m reaches (Figure 7), upstream of which the creek flows through a small, degraded wooden culvert under the railroad embankment. The culvert appeared to be a barrier due to its size and condition. Given the occurrence of the barrier culvert and safety concerns about crossing the railroad embankment, sampling of the creek was terminated at this point. No larval lampreys were detected in the two reaches sampled. Water temperature was 12.9°C, conductivity was 155.1 $\mu$S /cm, and % type 1 substrate was 50% in the two reaches sampled.

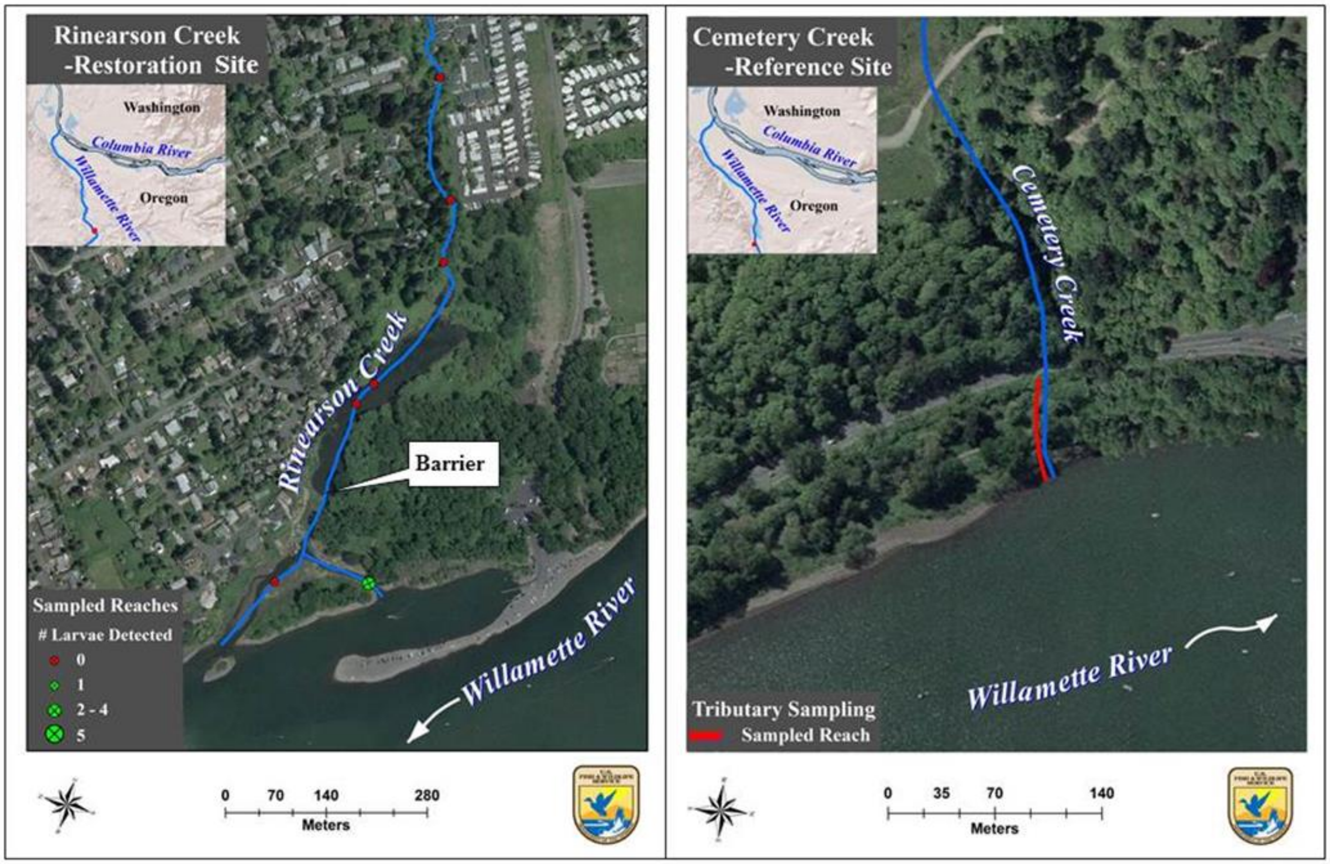


**Figure 7. Within the seven tributary reaches sampled in Rinearson Creek (above left) larval lampreys were detected in one reach (green point). In the approximately 100 m of stream sampled within Cemetery Creek (above right; red line), no larval lampreys were detected.**

**Figure 8. Electrofishing the lowermost GRTS sample reach in Rinearson Creek, approximately 30 m upstream of the confluence with the Willamette River. Three larval Pacific lampreys were collected in this reach. The morphology of the channel suggests backwater intrusion from the Willamette River into this segment of Rinearson Creek is common.**

## Conclusions

Both the Rinearson Natural Area restoration site and the Cemetery Creek reference site were found to be occupied by Pacific lamprey. All observed Pacific lamprey were of the larval life stage, no detections of juveniles or evidence of adults (i.e., spawning nests) occurred. All larvae collected appeared healthy based on visual observation of external features, no abnormalities or indications of disease or poor health were observed. Collected larvae occurred across a wide range of size classes (i.e., total length), and presumably comprised multiple age/year classes based on the observed differences in length.

At the Rinearson Natural Area, confluence habitats in the Willamette River adjacent to the mouth of Rinearson Creek, as well as one of seven tributary reaches in Rinearson Creek were occupied by larval Pacific lamprey. At the Cemetery Creek reference site, only confluence habitats in the Willamette River adjacent to the mouth of Cemetery Creek were occupied by larval Pacific lamprey; no larvae were detected in two tributary reaches within Cemetery Creek. The larvae detected in confluence habitats were likely to have originated in spawning areas of tributaries that enter the Willamette River upstream of the study areas (for example, the Clackamas River basin) and gradually dispersed downstream to their location of capture. Evidence suggesting dispersal of larval lamprey out of tributaries and into mainstem habitats has been observed previously in the mainstem Columbia River and Willamette River basins (Jolley et al. 2012; Jolley et al. 2013; Jolley et al. 2014) and may occur over extensive distances (Scribner and Jones 2002; Derosier et al. 2007). The presence of larvae in Rinearson Creek confluence habitats suggests newly created confluence habitats following restoration would also likely be suitable and available for colonization by larvae moving downstream in the mainstem Willamette River. Future sampling of confluence habitats following restoration would be warranted to monitor and evaluate the effects of restoration on larval lamprey occupancy in these habitats.

**Figure 9. Water diversion structure and impounded area on Rinearson Creek within the Rinearson Natural Area restoration site. The structure is a passage barrier to upstream migrating fish. Five of seven 50 m GRTS sample reaches in Rinearson Creek were located upstream of the barrier.**

In its current condition, natural production (adult spawning and larval rearing) of lamprey in Rinearson Creek appears unlikely given the impassable water diversion structure about 200 m from the Willamette River that limits fish usage to the lowermost segment of the creek. Suitable Pacific lamprey spawning and rearing habitats were scarce in the segment of creek between the barrier and Willamette River confluence. Larval Pacific lampreys were detected in one tributary reach below the barrier, however the reach was located about 30 m from the Willamette River, in a slough-like area that appeared to be significantly influenced by Willamette River backwater (Figure 9; presumably due to both tidal variation and changes in Willamette River discharge). These larvae were also likely to have originated in another tributary of the Willamette River and dispersed downstream into the location of capture in Rinearson Creek during periods of high discharge or high tide. Upstream of the barrier, potentially suitable adult spawning habitats as well as type 1 larval burrowing habitats occurred in the five reaches sampled, but no larvae were detected in any reach. Thus, removal of the water diversion structure as part of the restoration of Rinearson Natural Area would likely allow migratory fish such as adult Pacific lamprey (and adult western brook and river lamprey) to access and potentially recolonized suitable areas in Rinearson Creek. Future sampling of tributary reaches in Rinearson Creek following the removal of the passage barrier would be warranted to monitor and evaluate potential lamprey recolonization of the creek.

No *Lampetra* spp. larvae were observed among larvae large enough to be identified morphologically (i.e., those > 60 mm TL); whereas Jolley et al. (2012) reported 50-59% of larvae collected in the lower Willamette River were *Lampetra* spp. Here, some proportion of the larvae too small to identify morphologically could potentially be *Lampetra* spp. larvae. However, assigning genus identification to these larvae would require genetic methods to be used. Currently, funding for genetic identification of larvae is not available. Tissue samples collected from all larvae are archived at the CRFPO in the event funding becomes available at a future date.

Data contained in this report will serve as the baseline for pre- and post-restoration monitoring of the Rinearson Natural Area restoration site paired with the Cemetery Creek reference site. Similarities of confluence habitats at both locations should allow for comparisons of larval occupancy pre- and post-restoration and conclusions regarding the effects of restoration on larval lampreys to be proposed. Post-restoration sampling is anticipated to occur at Rinearson Natural Area in calendar year 2016 pending completion of restoration actions. In addition, post-restoration sampling at the Alder Point restoration site (Jolley et al. 2015) and its associated reference site (Ross Island) is also anticipated to occur in 2016 pending completion of restoration actions at Alder Point. The results of these investigations, along with any additional pre-restoration monitoring that occurs in calendar year 2016, will be summarized and reported in an annual report in spring of 2017.

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# Appendix 1.

Sediment descriptions from Rinearson Natural Area restoration and Cemetery Creek reference sites can be provided upon request.

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